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Vascular development of the bovine corpus luteum *in vitro*: characterization of endothelial cells and endothelial progenitor cells.

The corpus luteum is an excellent model of blood vessel development in the adult organism. Up to now there has been no explanation for the enormous angiogenic potential of this organ. Endothelial cell cultures of the bovine corpus luteum that were isolated in preliminary studies showed different angiogenic potency (high angiogenic to non angiogenic). The aim of the present study was a comparative investigation of these functionally heterogenous cultures on a morphologic and molecular level. It should also be elicited whether in the high angiogenic endothelial cultures potentially specialized cells, i.e. stem and progenitor cells, play a critical and initiating role during vascular development in the corpus luteum.

Research on adult stem and progenitor cells represents a relatively new terrain. Therefore a possible participation of these cells in the vascularization of the bovine corpus luteum has not been investigated so far. In the present work a strategy was developed for the identification of stem cells in the adult bovine. The immunocytochemical localization of stem cell markers as well as investigations at the transcriptional level via conventional RT-PCR were linked with the analysis of the specific growth pattern of cells on a light microscopic and ultrastructural level. Thereby it was essential to clarify the coexpression and kinetics of the expression pattern of stem cell markers used, i.e. CD31 (PECAM-1), CD34, VEGF-R1 (flt-1), VEGF-R2 (flk-1, KDR) and CD117 (c-kit, SCF-receptor).
In order to characterize systematically the heterogenous endothelial cell cultures the adhesion molecules CD29 and CD51/61 were demonstrated immunohistochemically. Morphology of cells was investigated by electron microscopy and histological methods. The screening of the cell cultures with the palette of methods used resulted in the following:

One of the cell cultures of the bovine corpus luteum in development can be regarded as new model of *in vitro* vasculogenesis. In this culture a high potency for the development of capillary like structures existed. The *in vitro* vascular development was initiated by a group of specific cells showing characteristic morphological features of progenitor cells. These cells, which were designated as “Starting Points”, proliferated intensely and grew cellular clusters.

After networking of the clusters, a plexus of capillary like structures was formed. Analysis of specific cells on an ultrastructural and molecular level provided evidence of their character as endothelial progenitor cells.

Investigations of two endothelial cell cultures of the corpus luteum in regression resulted in new findings on *in vitro* angiogenesis. Both cultures were arranged in capillary like structures in the framework of angiogenesis. However these cultures demonstrated different morphological phenotypes of angiogenesis indicating different angiogenic activity in early versus late stages of luteal regression. In both cultures cells expressing stem cell markers were detected. These findings suggest that specific endothelial subpopulations retain a plastic and immature nature.

In another culture of the corpus luteum cyclicum in regression cells were found that may potentially act as bipotent precursors of endothelial and granulosa cells. The cells of this culture persisted in a two-dimensional monolayer. Cellular and molecular analysis showed distinct parallels of attributes of endothelial as well as granulosa cells.

Lumenization of endothelial tubes so far has only been stated in models of angiogenesis. In this study different cellular mechanisms of vascular lumenization, like for example involvement of apoptosis, were described in endothelial cells that built capillary like structures. A fundamental difference in lumenization between the cultures of luteal development and regression became manifest. In the case of the vasculogenic cell culture of the corpus luteum in development the formation of tubular structures started in the cellular clusters of differentiated endothelial progenitors. This phenomenon was described for the first time.

During the phase of stabilization *in vivo* in most vessels an attachment of pericytes can be seen. An interesting finding of the present study was the segmental appearance of a second cellular layer around the tubular structures during long term cultivation. This cellular layer could possibly represent pericyte-like cells.

Immunocytochemical labelling of adhesion molecules clearly showed three-dimensionality of vascular structures *in vitro*. The communication between endothelial cells and between endothelial cells and the extracellular matrix as well as the synthesis of an own three-dimensional substrate were visualized. A particular finding of this dissertation observed in luteal angiogenic cultures was the determinated architecture of vascular development. Despite initial heterogeneity three dimensional networks of capillary like structures were established that corresponded to the architecture of the capillary bed in the corpus luteum *in vivo*. This observation that was described for the first time in this study, proves that *in vitro* models are similar

to the situation *in vivo* and therefore can be used as substitute and supplement methods to animal experiments.